

Amendments to the Specification:

Please amend the paragraph bridging lines 19 and 25 on page 3 in the following manner:

The level of nucleotide homology can be determined with the computer program "BLAST 2 SEQUENCES" by selecting sub-program: "BLASTP," ~~that can be found at~~
~~www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html~~. A reference for this program is Tatiana A. Tatusova, Thomas L. Madden FEMS Microbial. Letters 174: 247-250 (1999). Parameters used are the default parameters: Reward for a match: +1. Penalty for a mismatch: -2. Open gap: 5. Extension gap: 2.
Gap x_dropoff: 50.

Please amend the paragraph bridging lines 21 and 27 on page 7 in the following manner:

The level of protein homology can be determined with the computer program "BLAST 2 SEQUENCES" by selecting sub-program: "BLASTP," ~~that can be found at~~
~~www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html~~. A reference for this program is Tatiana A. Tatusova, Thomas L. Madden FEMS Microbiol. Letters 174: 247-250 (1999). Matrix used: "blosum62". Parameters used are the default parameters: Open gap: 11. Extension gap: 1. Gap x_dropoff: 50.

Please amend the paragraph bridging lines 14 and 28 on page 11 in the following manner:

Methods for large-scale production of antibodies according to the invention are also known in the art. Such methods rely on the cloning of (fragments of) the genetic information encoding the protein according to the invention in a filamentous phage for phage display. Such techniques are described i.a. ~~at the "Antibody Engineering Page" under "filamentous phage display" at~~
~~http://aximtl.imt.uni-markburg.de/~rek/acpphage.html~~, and in review papers by Cortese, R.

et al., (1994) in Trends Biotechn. 12: 262-267., by Clackson, T. & Wells, J.A. (1994) in Trends Biotechn. 12: 173-183, by Marks, J.D. et al., (1992) in J. Biol. Chem. 267: 16007 16010, by Winter, G. et al., (1994) in Annu. Rev. Immunol. 12: 433-455, and by Little, M. et al., (1994) Biotechn. Adv. 12: 539-555. The phages are subsequently used to screen camelid expression libraries expressing camelid heavy chain antibodies. (Muyldermans, S. and Lauwereys, M., Journ. Molec. Recogn. 12: 131-140 (1999) and Ghahroudi, M.A. et al., FEBS Letters 414: 512-526 (1997)). Cells from the library that express the desired antibodies can be replicated and subsequently be used for large scale expression of antibodies.

Please amend the paragraph bridging lines 8 and 17 on page 14 in the following manner:

Vaccines according to the present invention may in a preferred presentation also contain an immunostimulatory substance, a so-called adjuvant. Adjuvants in general 10 comprise substances that boost the immune response of the host in a non-specific manner. A number of different adjuvants are known in the art. Examples of adjuvants frequently used in cow vaccines are muramyl dipeptides, lipopolysaccharides, several glucans and glycans and ~~Carbopol~~^(R) **CARBOPOL[®]** (~~[[a]] an acrylic~~ homopolymer). The vaccine may also comprise a so-called "vehicle". A vehicle is a compound to which the protein adheres, without being covalently bound to it. Such vehicles are i.a. bio-microcapsules, micro-alginates, liposomes and macrosols, all known in the art.

A special form of such a vehicle, in which the antigen is partially embedded in the vehicle, is the so-called ISCOM (EP 109.942, EP 180.564, EP 242.380) In addition, the vaccine may comprise one or more suitable surface-active compounds or emulsifiers, e.g. ~~Span or Tween~~ **SPAN[™] or TWEEN[™]**.

Please amend the paragraph bridging lines 3 and 32 on page 19 in the following manner:

EXAMPLE 1

Cloning and expression of the gene encoding the 22.5 kDa S. uberis protein

The amino acid sequence of the 22.5 kDa protein according to the invention contains a hydrophobic N-terminal signal sequence of 21 residues (SEQ ID 2). For expression in *E. coli* this domain was deleted and an expression construct was made in a pET-derived vector pETHis1 (ampicillin-resistance), which was constructed using a standard pET-vector as known in the art, into which i. a. several histidines were introduced. Figure 1 **SEQ ID No: 5** gives the sequence of the relevant region comprising a T7-promoter and several histidines at the 5'-and 3'-end of the multicloning site.

The DNA fragment encoding residues 22-200 of the 22.5 kDa protein was amplified by PCR using a forward primer (CATgCCATgggg**CATATgTATATAACACATCAAAATgTAC**), **SEQ ID No: 3** that started at residu 22 (codon underlined) with an additional *NdeI* restriction site (bold italics) and a reverse primer (gCggg**ATCCAAATTTagATAATAATTgTATg**) **SEQ ID No: 4** that contained the last 22 nucleotides of the gene (underlined), lacking the TAA stop codon but with an additional *BamHI* restriction site. Oligonucleotides were purchased from Gibco (BRL Life Technologies Inc., USA). This cloning strategy results in an expression product with a 6xHIS tag at the N-terminus and a 10xHIS tag at the C-terminus, which could be efficiently used for purification of the protein by means of metal affinity columns.

PCR amplification was performed using a PE GeneAmp PCR system 9700 (Perkin Elmer, California, USA). The PCR mixture consisted of 20 U/ml **Supertaq SUPERTAQ™**, 1 x **Supertaq SUPERTAQ™** (**Taq polymerase isolated from *E. coli***) buffer, 80 µM (each) of dATP, dCTP, dGTP, dTTP (HT Biotechnology, Ltd., Cambridge, UK), 10 pmoles of the used primers and 1 µl of the chromosomal DNA of *S. uberis* as a template in a total volume of 50 µl. PCR was performed using the following program: denaturation for 2 minutes at 95°C, followed by 30 cycles consisting of 30 seconds denaturation at 95°C, 30 seconds annealing at 45°C and 1 minute elongation at 72°C, ending with 7 minutes 72°C and cooling down to 4°C.

The size of this PCR fragment was confirmed by agarose gel electrophoresis and the band was excized from gel and purified using the **Qiaquick QIAQUICK™** gel (**silica gel membrane that**

binds DNA) extraction kit (Qiagen, Inc. CA, USA).

Please amend the paragraph bridging lines 1 and 10 on page 20 in the following manner:

system 9700 (Perkin Elmer, Ca, USA). The cycle sequencing reaction mix consisted of approximately 375 ng of miniprep DNA or approximately 75 ng PCR-product (PCR primers should be removed), 8 μ l ~~Big-dye-Terminator-Ready-Reaction-mix~~ **BIG DYE TERMINATOR READY REACTION MIX™** (colored nucleotides and corresponding enzymes) (Perkin Elmer, Ca, USA), 2.5 pmol primer in a total volume of 20 μ l. Cycle sequence reaction was performed using the following program; 25 cycles with 10 seconds 95°C, 5 seconds 50°C and 4 minutes 60°C and then 4°C. The unincorporated dyes were removed by the ~~Dye-Ex-Spin-Kit~~ **DYE EX SPIN KIT™** (dye terminator removal kit) (Qiagen, Inc. CA, USA). The nucleotide sequences were determined using an ABI 310 Automatic Sequencer. Sequence analysis was performed using sequencher version 4.0.5 (Gene Codes Corporation, Michigan, USA).

Please amend the paragraph bridging lines 11 and 25 on page 20 in the following manner:

Plasmid DNA of correct pETHis1-USP22.5 clones was isolated and transformed into *Escherichia coli* host strain ~~BL21-Star~~ **BL21 STAR™** (DE3) containing vector pLysS [genotype: F. ompT, hsdS_B (r_h⁻, m_h⁻), gal, dcm, mc131 (one shot chemically competent E. coli bacterial strain) (DE3) pLysS (Cam^R). *E. coli* strain ~~BL21-Star~~ **BL21 STAR™** (DE3) containing the plasmid pLysS and pETHis1-USP22.5 was grown overnight at 30°C or 37°C and 200 rpm in 5 ml ~~Terrific-Broth~~ **TERRIFIC BROTH™** (tryptone, yeast and glycerol culture medium) (Sambrook et al. 1989. Molecular Cloning; a laboratory manual. Cold Spring Harbor Laboratory. Cold Spring Harbor, N.Y.) with 100 μ g/ml ampicillin, 25 μ g/ml chloramphenicol and 5 mM MgSO₄.

This overnight culture was diluted 1:50 in 50 ml of the same fresh medium.

These cultures were grown under the same conditions until the OD₆₀₀ had reached 0.5, 20

measured at 600 nm on a ~~NovaspecH~~ NOVASPEC II™ spectrophotometer (Pharmacia). At this point a 100 µl sample was taken for analysis. At OD₆₀₀ of 0.5 the cultures were induced with IPTG to a final concentration of 0.1 mM and continued to grow for a subsequent 3 hours. Again, a 100 µl sample was taken for analysis. The samples were analysed on a NuPAGE™ electrophoresis System by SDS-PAGE. The gels were stained with Coomassie Brilliant Blue (CBB).